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(54) AUXILIARY AGENT COMBINATION FOR IMPROVING MICROBIOLOGICAL METHODS

(57) The invention relates to an auxiliary agent combination for improving microbiological methods, in particular aerobic processes for the obtaining of microbial protein. For improving the oxygen transfer capacity in the aerobic growing of microorganisms for the obtaining of protozoal protein on the basis of hydrocarbons, as regards the lowering of the high energy input and the input of apparatuses, there is not yet a satisfactory solution of the problem. According to the state of the art, surfactants or surfactant combinations are added to the fermentation liquid for improving the oxygen transfer capacity. It was surprisingly found that by the addition of an auxiliary agent combination of the known non-ionogenic surfactant family of ethylene oxide - propylene oxide adducts having an average molecular weight of 1200-3500, and the non-surfactant family of the polyethylene glycols having an average molecular weight of 150-5000, an increase of the concentration of biomass of about 20 % can be achieved. - Figure -

Title of the invention

Auxiliary agent combination for improving microbiological methods

Field of application of the invention

The invention relates to an auxiliary agent combination for improving microbiological methods, in particular aerobic processes for the obtaining of microbial protein. It lies predominantly in the field of technical microbiology. The invention can yet be employed also in other fields in which oxygen is to be transferred from the air into an aqueous medium.

Characteristics of the known technical solution

Numerous aerobic fermentation methods for growing microorganisms with the aim of obtaining cellular bodies as well as the obtaining of biochemical reaction products are known.

Typical examples for this are large-scale methods of the aerobic growing of microorganisms for the obtaining of protozoal protein on the basis of carbohydrates and hydrocarbons as the carbon source.

These aerobic methods are all characterized by high oxygen requirement of the fermentation process.

Generally, the oxygen requirement is met by the delivery of air and deep dispersion into the aqueous fermentation medium by means of various technical equipment. The supply of oxygen of the fermentation process is, thus, a process requiring high input of energy and apparatuses.

For improving the oxygen transfer capacity of these technical systems, non-ionogenic surfactants or surfactant combinations are added to the aqueous fermentation liquid. To this end, the state of the art is described in the following patent documents:

(1) According to DD patent document No. 96722, addition products of ethylene oxide and lower aliphatic alcohols or diols as well as alkylene carboxylic acid methyl taurides are used.

(2) According to DD patent document No. 104,099, a combination of a non-ionogenic surfactant with an anion-active surfactant is recommended as being particularly favorable. Here, the non-ionogenic surfactant is a propylene - ethylene oxide adduct having 25-20 molecules of propylene oxide and 20-25 molecules of ethylene oxide. A defined alkyl sulfonate is employed as anion-active surfactant.

(3) Moreover, according to DT publication No. 1,442,296 of the Socony Mobil Oil Inc., it is known that addition products of ethylene oxide with fatty alcohols, fatty amides, alkyl and aryl groups as well as particular fatty acid esters may be employed.

(4) For improving the contact between microorganisms and the droplets of liquid alkanes, according to DT publication 23 29 811, various polyoxypropylene glycol ethers are suggested for methods of hydrocarbon fermentation.

(5) In DT publication No. 25544625, for improving the growing and separation of microorganisms, only an addition product of ethylene oxide and propylene oxide is used in the fermentation of crude oil distillate fractions.

In this method, in combination with other method steps, this auxiliary agent is simultaneously utilized for the separation and for the favorable influencing of the purification of the biomass.

This presented state of the art has the following disadvantages:

In cases (1), (3) and (4), different families such as ethylene oxide, propylene oxide as well as the respective basis components (alcohol, acid, amides, and so on) must be available. These circumstances cause production technologies requiring high input of substances and apparatuses.

Although a basic auxiliary is employed in the method according to (2), which is solely produced from ethylene oxide and propylene oxide, the best effect both as a fermentation auxiliary and as a separation auxiliary is yet only achieved in combination with a strongly anionic class of surfactants of the alkyl sulfonates.

In the method according to (5), solely an ethylene oxide and propylene oxide adduct is used for the fermentation and separation. However, as apparent from the broad examined ranges and the relatively high employed concentrations according to this document, the performance limit of the ethylene - propylene oxide adducts has been reached and exhausted in this case.

Object of the invention

An object of the present invention is to overcome the described disadvantages according to (1) - (4) and to further increase the performance capability of the ethylene oxide - propylene oxide adducts according to (5) without employing new basic substances for the production of the auxiliary.

Explanation of the essence of the invention

The technical object of the invention consists in that the inner structure of the aqueous culture medium, in the presence of an ethylene oxide - propylene oxide adduct, is to be influenced to the effect that better conditions for the substance exchange gas – liquid - microorganisms are achieved without the occurrence of blockages of the interfaces.

Surprisingly, it was found that this object can be attained by the addition of polyethylene glycol. Polyethylene glycol can easily be produced from ethylene oxide.
Polyethylene glycol does not belong to the family of surfactants. The achieved positive effect in combination with the non-ionic surfactants of the propylene - ethylene oxide family is attained by an optimal influencing of the inner structure of the aqueous culture liquid as a consequence of a synergistic action of the hydrate shells both at the ethylene oxide - propylene oxide adduct and at the polyethylene glycol.

As is shown in the following examples, the positive effect of this auxiliary combination consists in that, upon the additional presence of the polyethylene glycol having an average molecular weight of 150-5000, an increase of the oxygen transfer capacity is achieved or a lowering of the requirement of ethylene oxide - propylene oxide adduct is attained for the fermentation or according to method (5) also for the separation.

Examples

The method according to the invention is illustrated by means of the following examples:

Example 1

For the determination of the improvement of the oxygen transfer coefficient by means of the auxiliary agent combination, the following experiments were performed:

The experimental principle consisted in that 100 mg/l each of the respective auxiliary agent combinations were added to water samples, which had been rendered oxygen-free with Na_2SO_3, and that the reconcentration time ("Aufsättigungszeit") with oxygen of this solution was measured by means of a pO_2 measuring probe under defined conditions, i.e. standardized surface aeration.

The measured time was put into the ratio to the reconcentration time of the pure water sample, i.e. the water sample not containing a surfactant and being free of oxygen. It was

found that the reconcentration times (50 % value), which upon addition of 100 mg/l propylene oxide - ethylene oxide in a molecular weight range of 1200-3500 were 40-60 % of that of the pure water, could surprisingly be decreased to 10-30 %, when polyethylene glycol was contained in these adducts in a molecular weight range of 150-5000.

Example 2

The measurement of the saturation time ("Sättigungszeit") via surface aeration can, however, only be an indication for the efficacy of auxiliary agents in technical fermentation processes. As is known, a linear improvement rate, analogous to the surface aeration, is not achievable in technical aerobic fermentation processes, since essential additional factors become effective, e.g.

- limited specific aeration rate of the fermentation mixtures due to foaming or reaching the flooding point
- influences of the microorganisms and the metabolic products.

For this reason, an improvement of the effect was verified by means of a method example according to (5). According to this method, *Candida guilliermondii* is grown on crude oil distillate and is separated with the same auxiliary agent from the crude oil distillate for recovering microbial protein. For this purpose, *Candida guilliermondii* was continuously grown in a known manner in a 500 l fermenter.

0.1 g/l of the known ethylene oxide - propylene oxide adduct having an average molecular weight of 2100, which contained 5.2 % polyethylene glycol having an average molecular weight of 380, was added to the fermentation medium. The achieved improved biomass concentrations as compared to the growing without auxiliary agent addition are given in the following overview.

The specific flow rate and aeration rate, the performance entry by the stirring unit and the dosage of the nutrient and trace components were kept constant over all experiments.

Specific aeration rate	=	100 l air/1 kg fermentation liquid and hour
Specific performance entry	=	2.3 W/ kg fermenter liquid
Specific flow rate	=	0.2 h^{-1}

without auxiliary	9.6 g	yeast dry substance/ kg fermenter liquid
with ethylene oxide - propylene oxide adduct	10.1 g	yeast dry substance/ kg fermenter liquid
with ethylene oxide - propylene oxide adduct + polyethylene glycol	12.3 g	yeast dry substance/ kg fermenter liquid

Applying the auxiliary mixture according to the invention, an increase of the biomass concentration by about 20 % was, thus, obtained.

Another 300 mg of the above-mentioned ethylene oxide - propylene oxide adduct + polyethylene glycol were added to the fermentation mixture which was continuously withdrawn from the fermenter, it was heated to 80 °C and the crude oil distillate was removed to a residual content of 0.6 % in the yeast phase in a continuously working separator.

Therefore, also during oil separation, an improved effect of the auxiliary agent combination was attained in the range of low concentrations.

Analogously good results were attained in further experiments in which auxiliary agent combinations having a polyethylene glycol content of up to 20 percent by weight and molecular weights of up to 5000 were used.

Claim of the invention

Auxiliary agent combination for improving microbiological methods, in particular, of microbial protein, characterized in that the auxiliary agent combination consists of a mixture of the known non-ionogenic surface-active family of the ethylene oxide - propylene oxide adducts having an average molecular weight of 1200-3500 and the non-surfactant family of the polyethylene glycols having an average molecular weight of 150-5000, wherein the non-surfactant polyethylene glycol is employed in an amount of 0.5-20 percent by weight based on the ethylene oxide - propylene oxide adduct.